Technical Manual

Citric Acid (CA) Colorimetric Assay Kit

- **Catalogue Code: MAES0179**
- **Size: 96T**
- **Research Use Only**

1. Key features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

0.06-2.0 mmol/L

Sensitivity:

0.06 mmol/L

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. Background

In biochemistry, citric acid is an intermediate in the citric acid cycle and plays an important role in metabolism. Citric acid levels in blood and urine are affected by factors such as age, gender, diet, citric acid precursors, and parathyroid hormone and sex hormones.

3. Intended Use

This kit can be used to measure citric acid (CA) content in animal tissue, serum (plasma) and mitochondria samples.

4. Detection Principle

In acidic condition, Cr (Ⅵ) will be reduced to Cr^{3+}, Cr^{3+} reacts with citric acid. And the product has a characteristic absorption peak at 545 nm, therefore the content of citric acid in sample can be calculated by measuring the absorbance value at 545 nm.

5. Kit components & storage

Item	Specification	Storage
Extracting Solution	60 mL × 2 vials	2-8°C, 6 months
Lysis Buffer	20 mL × 1 vials	2-8°C, 6 months
Reducing Agent	Powder × 1 vial	2-8°C, 6 months, shading light
Chromogenic Agent	1.2 mL × 2 vials	2-8°C, 6 months, shading light
CA Standard (2 mmol/L)	2 mL × 1 vial	2-8°C, 6 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (535-555 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Normal Saline (0.9% NaCl)
- PBS (0.01 M, pH 7.4)

6. Assay Notes:

There should be no bubbles in the wells of the microplate when measuring the OD value.

7. Reagent preparation:

1. Bring all reagents to room temperature before use.

2. Preparation of **working solution:** Dissolve a vial of reducing agent with 5 mL of extracting solution fully. The prepared solution can be stored at 2-8°C with shading light for 7 days.

8. Sample Preparation

Sample requirements: The samples could not contain chelating agents such as EGTA and EDTA, or reductive substances such as DTT and mercapto ethanol.

1. Liquid sample:
Take 0.1 mL of liquid sample and add 0.9 mL of extracting solution, mix fully. Centrifuge at 11000 g for 10 min at 4°C, then take the supernatant and stand on ice for measurement.

2. Tissue sample:
Take 0.1 g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C. Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of extracting solution (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4°C. Take the supernatant to preserve it on ice for detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

3. Mitochondria:
Take 0.1 g tissue, add 0.9 mL of extracting solution, then homogenize the sample in ice water bath. Centrifuge at 600 g for 10 min at 4°C, then take the supernatant to another EP tube and centrifuge at 11000 g for 10 min at 4°C, discard the supernatant (This supernatant can be used for the determination of citric acid content in cytoplasmic). Add 200 μL of lysis buffer and dissolve the precipitate fully with vortex mixer. Centrifuge at 11000 g for 10 min at 4°C, then take the supernatant and stand on ice for measurement.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (0.06-2.0 mmol/L).

The recommended dilution factor for different samples is as follows (for reference only).

Sample Type:	Dilution Factor
Human serum	5-20
Dog serum	5-10
Rat serum	10-20
Horse serum	5-10
Mouse plasma	5-20
10% Rat brain tissue homogenate	5-10
10% Rat liver tissue homogenate	5-20
10% Rat kidney tissue homogenate	5-10
10% Rat lung tissue homogenate	15-30
10% Mouse heart tissue homogenate	5-20

Note: The diluent is extracting solution;

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 545 nm

Plate Set Up:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H, standard wells; S1-S80, sample wells.

10. Operation Steps

The preparation of standard curve

Dilute standard (2 mmol/L) with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 2.0, 1.5, 1.2, 1.0, 0.8, 0.5, 0.2, 0 mmol/L.

The measurement of samples

1. **Standard well:** Take 20 μL of standard solution with different concentrations into the corresponding wells.
   Sample well: Take 20 μL of sample into the corresponding wells.

2. Add 140 μL of extracting solution to each well.

3. Add 20 μL of working solution to each well.

4. Add 20 μL of chromogenic agent to each well.

5. Mix fully with microplate reader for 5 s and stand at room temperature for 30 min. Measure the OD values of each well at 545 nm with microplate reader.

Operation Table

	Standard well	Sample well
Sample (μL)		20
Standards solution with different concentrations (μL)	20	
Extracting solution (μL)	140	140
Working solution (μL)	20	20
Chromogenic agent (μL)	20	20
Mix fully with microplate reader for 5 s and stand at room temperature for 30 min. Measure the OD values of each well at 545 nm with microplate reader.		

Note: If there is obvious turbidity in the well after reaction for 30 min, dilute the sample and test again.

11. Calculations

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: y= ax + b.

1. Serum (plasma) and other liquid sample:

$$\text{CA content (mmol/L)} = (\triangle A - b) \div a \times f \times 10$$

2. Tissue sample:

$$\text{CA content (μmol/g wet weight)} = (\triangle A - b) \div a \times f \div \frac{m}{V}$$

3. Mitochondria sample:

$$\text{CA content (mmol/gprot)} = (\triangle A - b) \div a \div C_{pr} \times f$$

y: $OD_{Standard}$ – OD_{Blank} (OD_{Blank} is the OD value when the standard concentration is 0)

x: The concentration of standard

a: The slope of standard curve

b: The intercept of standard curve

ΔA: OD_{Sample} - OD_{Blank}

10: Dilution factor of liquid sample in extraction step (0.1 mLof sample + 0.9 mL of extracting solution)

f: Dilution factor of sample before test

m: The weight of tissue sample (0.1 g)

V: The volume of extracting solution (0.9 mL)

C_{pr}: Protein concentration of sample (gprot/L)

12. Performance Characteristics

Detection Range	0.06-2.0 mmol/L
Sensitivity	0.06 mmol/L
Average recovery rate (%)	95
Average inter-assay CV (%)	4.0
Average intra-assay CV (%)	4.0

Analysis

Take 0.1 mL of human serum and add 0.9 mL of extracting solution, mix fully and extracted by vortex for 1 min. After centrifugation, the supernatant was diluted for 20 times with extracting solution, and take 20 μL to the corresponding well, carry the assay according to the operation table.

The results are as follows:

Standard curve: y = 0.0672 x + 0.0009, the OD value of the sample is 0.099, the OD value of the blank is 0.071, and the calculation result is:

CA content (mmol/L)=（0.099-0.071-0.0009）÷ 0.0672 × 20 × 10 = 80.65 mmol/L

Safety Notes

Some of the reagents in the kit contain dangerous substances. Avoid touching skin and clothing.

Wash immediately with plenty of water if touching it carelessly.

All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.

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